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MANUSCRIPT REPORTS OF THE EXPERIMENTAL STATIONS

No. 32

Title

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HALIFAX, 1930

BIOLOGICAL BOARD OF CANADA

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THE EFFECT OF SALT SOLUTIONS ON THE WEIGHT OF CODFISH MUSCLE.

By H. Ritchie Chipman.

In earlier investigations at this Station the effect of salt solutions upon the weight of fish muscle had been reported. In 1925, J. C. Forbes[1] noted some weight changes taking place during the salting of fish under different conditions; and in 1928 R.F. Ross[2] showed the weight changes of pieces of muscle when soaked in salt solutions of various concentrations. The present investigations developed from some experiments on salt penetration which were being carried out at the same time as Dr. Ross was investigating muscle shrinkage, and show the effect of various ions in the salt solutions upon the weight of the muscle.

Method of Procedure.

Pieces of muscle were cut from the fillet and weighed on a watchglass to 0.002 grams. They were then placed in 100 cc. of the solution in a small "Perfect Seal" jar and allowed to remain for the required time in a thermostatically controlled box at 10°C. The pieces were then removed from the solutions by means of forceps, allowed to drain for fifteen seconds and then weighed on a watchglass .

In adopting this method of handling some objection could be raised on account of the amount of liquid which remained on the surface of the piece of fish and which was included in the weight. After soaking in some of the solutions, the fish became either brittle or slimy, and any attempt to dry off the surface by means of cheesecloth or filter paper would cause a loss of material. It was therefore felt that it was better to keep to the same method in all cases in spite of the error which was sometimes introduced. In every case, however, the piece of fish was dipped in the solution and allowed to drain for the fifteen seconds before the initial weighing so that a "wet" weight was recorded.

Some variations were found which could not be explained in any way. In some cases pieces cut from fillets which appeared to be similar and which had been handled in exactly the same manner showed differences in percentage weight changes. In order to get uniformity of material the pieces were either cut from the same fillet, or, if that were not possible, from fillets obtained at the same time and place. The pieces were so cut that they were approximately of the same shape and weight.

Unless otherwise stated all salts were of C. P. quality, and distilled water was always used. The solutions were made up to an accuracy of at least 1%. On account of the very wide variations which were encountered no statement of accuracy can be made, but it was found that when repeat experimants were performed the relative results were the same even though the actual figures were quite different.

The effect of the duration of storage.

Some preliminary investigations of weight changes showed that the duration of storage of the fish did not cause any great variation, but an experiment was carried out to see if it were so. A fish which had been dead only a couple of hours was filletted and pieces of the muscle placed in sealed jars at different temperatures for forty eight hours. The different temperatures would produce various degrees of autolysis which would correspond to different times of storage. A small crucible of water was placed in each jar to keep the air saturated with water. The jars were placed in thermostatically controlled boxes at 0°, 10°, 21°, and 35° C. After twenty four hours the piece stored at 35° was fund to be too rotten to use and it was discarded. After the others had remained for forty eight hours they were removed and placed in saturated sodium chloride solutions. The jars were stored for twenty four hours at 10° C and the weight loss determined.

Table 1.

Temperature of storage C°	Percentage loss of weight.
0	13.28
0	14.56
10	10.82
10	13.89
21	13.22
21	14.13

The results of this experiment indicated that the duration of storage of the fish did not cause an appreciable change in the weight loss. The fish used was therefore obtained from local firms as desired.

The Effect of concentrated solutions.

When fish muscle is soaked in saturated solutions of sodium chloride there is a resultant loss of weight which rapidly assumes large proportions. This is shown by the following experiment where pieces of muscle were soaked in saturated solutions of Malagash, Turk's Island, and C. P. sodium chloride. The solutions were kept saturated by means of a layer of solid salt on the bottom of each jar and the jars were occasionally shaken to stir the solutions. The results are shown in Table 2.

Table 2.

Percentage weight loss

Period of soaking in hours	Kind of salt		
	Malagash	C.P.	Turk's Island.
0.25	0.4	1.1	1.7
0.5	0.4	2.0	3.4
0.75	1.7	2.9	4.7
1.25	3.3	4.1	6.4
2.0	5.5	6.2	8.7
4.0	9.1	9.6	12.9
5.0	10.0	10.7	14.9
6.0	11.6	11.7	16.0
7.0	12.6	12.2	17.0
23.0	14.4	15.2	22.2
29.0	17.0	16.4	24.2
94.0	18.9	17.6	29.0

It will be seen that the fish soaked in Turk's Island salt solution showed a greater percentage loss than those soaked in the other solutions. The reason for this was apparently the presence of calcium and magnesium in the Turk's Island salt.

The presence of calcium and magnesium in fishery salts has been shown by Tressler[3] and Taylor[4] to retard the penetration of sodium chloride into the muscle. On this account it would be expected that there would be an effect on the loss of weight.

Pieces of fish were then soaked in saturated solutions of sodium chloride to which were added amounts of calcium chloride, magnesium chloride and sodium sulphate so calculated as to be of a strength of 0.5% of the metal ion in 100 cc. of solution. Two series were run and the results shown in Table 3.

Table 3.

Percentage weight changes

Time in hours	Ions added to sodium chloride solution			
	None.	Calcium.	Magnesium.	Sulphate.
21	3.6	14.9	13.7	12.4
45	---	15.1	17.8	11.4
99	12.5	21.2	21.5	10.3
192	11.6	23.0	23.5	10.5
23	8.6	13.4	12.2	4.1
47	11.0	17.8	19.4	7.6
119	8.4	16.2	16.7	8.2
284	11.5	19.2	26.1	4.4

These figures are not very consistent, especially those obtained with the sodium chloride alone, but it may be seen that the addition of the ions of calcium and magnesium tend to increase the loss of weight. The effect of the sulphate ion is uncertain but it is believed that it does not have much effect. Other experiments with sodium sulphate showed either a diminishing of the weight loss or no change at all.

The results obtained with concentrated solutions are those of experiments which were not done during the past year. They are included in this report to give further information on the effect of salt solutions on the weight of the muscle.

The effect of dilute solutions.

According to the theories of Tressler[5] and Taylor[6], when a piece of fish muscle is soaked in salt solutions, osmosis will take place, and, if the concentration of the salt in the solution be greater than that of the salt in the muscle, water will be withdrawn from the fish. The salt concentration of fish muscle is about 1%, so that if osmosis alone is acting, it would be expected that a piece of muscle would gain in weight when immersed in pure water on account of the osmotic action of the salt in the muscle. The addition of salt to the water in which the fish is soaked should therefore lessen the amount of water taken up by the fish; and at the point where the salt concentration in the water is equal to the salt concentration in the muscle no water should be taken up and there should be no change in weight. Any further increase in the concentration of the solution surrounding the muscle should result in a reduction in weight due to the water being removed from the fish.

It was found that fish muscle does not behave in this manner. When the muscle is immersed in water it does gain in weight (about 30% in twenty four hours) as would be expected; and the addition

of salt to the water does produce a loss in weight. When the isotonic point is reached the weight is lower than it was in pure water, but there is still a considerable increase over the initial weight, showing that a lot of water has been retained by the muscle. As the concentration of the soaking solution increases beyond the isotonic point the weight of the muscle increases and reaches a maximum when the salt concentration is about Normal. An increase in salt concentration beyond normal results in a decrease in weight, until finally the weight is less than the weight of the muscle before soaking. This is shown by Table 4 which gives the results of an experiment in which pieces of muscle were soaked in various concentrations of sodium chloride for twenty four hours.

Table 4

Normality sodium chloride	Percent weight change
0.00	30.0
0.17	18.9
0.53	41.4
0.86	45.3
2.66	29.7
3.48	11.0
4.36	0.2
5.26	-10.7

These results are shown in Figure 1.

All the experiments in soaking in dilute solutions gave curves similar to that shown in Figure 1. The actual values vary considerably but the shape of the curve is always the same. It will be noticed that the weight of the fish at the minimum point is quite a bit greater than the original weight, (which is represented by 0% weight change).

To find out if the maximum and minimum points were to be found at any definite time after immersion, the experiment was repeated with the weighings made at different times. The results are given in Table 5. It will be seen that at every period of time the dip was present and the curves are all similar. The shape of the curve is therefore dependent upon the concentration and not upon the time of immersion.

Table 5.

Percentage weight changes

Salt Normality	1 hr.	2 hr.	4 hr.	5 hr.	6 hr.	24 hr.
0.00	9.6	13.3	19.4	21.2	26.9	31.3
0.15	6.8	9.0	12.6	14.0	15.2	20.9
0.53	12.1	17.1	23.7	25.4	28.3	47.6
1.33	13.4	19.9	26.3	29.4	31.2	43.0
2.68	6.4	8.9	10.9	12.1	14.2	28.7
4.00	1.7	1.2	0.8	0.8	0.3	4.8
5.34	- 3.3	-5.8	-9.1	-9.8	-10.6	-10.9

Experiments were carried out to see if similar curves would be obtained when other salts than sodium chloride were used. The concentrations did not exceed 1.2 N except in a few cases. The curves obtained were all similar. The results are given in Table 6.

Table 6.

Percentage weight changes.

Salt	Normality of salt solution						
	0.0	0.15	0.30	0.60	1.25	2.5	5.0
Sodium Chloride	29.2	17.9	22.0	42.2	---	--	--
" "	30.3	21.5	25.1	42.4	41.5	-	-
" "	26.2	--	19.0	41.0	34.6	20.6	-5.0
Potassium Chloride	29.2	16.7	21.7	35.4	---	--	--
" "	26.2	--	22.0	44.3	37.6	25.3	--
Calcium Chloride	29.2	-6.3	0.0	9.7	---	--	--
" "	26.2	--	1.7	8.0	1.4	-9.4	-26.0
Barium Chloride	29.2	2.9	15.6	23.2	---	---	--
Magnesium Chloride	29.2	9.2	18.6	37.6	---	---	---
" "	26.2	--	20.5	29.6	22.4	6.7	-15.5
Sodium Sulphate	30.3	22.1	23.6	43.6	23.8	--	---
Lithium Sulphate	22.7	13.8	37.5	35.4	14.0	--	---
Ammonium Sulphate	30.3	24.5	11.6	22.6	58.8	--	---

It will be seen from these results that the curves are similar. They are not shown in a figure as it would be too confusing. The minimum point occurs at the same concentration in almost every case. The figures are not conclusive enough to attribute any variation to the valency of the metal ion, but it will be noticed that in general a bivalent cation gives a smaller weight change at the maximum and minimum points.

It was thought useless to carry out experiments with other salts with bivalent or even trivalent cations as the metals would produce changes in the proteins which might mask the desired effect.

When a piece of fish muscle is immersed in water the resulting increase in weight is what one might expect from osmosis. Similarly the decrease from the weight produced by pure water when small amounts of salt are added can also be explained by osmotic action. This does not however give any reason for the rise in weight to a maximum at a salt concentration of 1 N nor for the decrease in weight which results when still stronger salt solutions are used.

An explanation of the shape of the curve can be found in the amphoteric character of the proteins and their solubility. According to Loeb (5) proteins act as amphoteric substances and are thus capable of combining with acids and bases depending on the pH. The protein will be ionized to some extent and as there is a non-diffusible ion present the Donnan membrane equilibrium will result in a diffusion of ions into the cells of the muscle. The solubility of fish muscle proteins was investigated by J. F. Logan (6) and it was found that the solubility increased with increasing salt concentration until a maximum was reached at about 1 N. after which a further increase of salt concentration produced a slight decrease in the solubility.

As the concentration of the salt solution in which the fish is immersed is increased beyond the point at which the dip in the curve is shown the solubility of the protein begins to increase and more water is taken into the muscle. This process goes on until the maximum solubility is reached. In the meantime as the salt concentration has been increasing the osmotic force becomes great enough to counteract the effect of the solubility and less water enters the muscle. This results in a smaller and smaller weight change until finally a point is reached where the forces are balanced and there is no change in weight on soaking. A still further increase in salt concentration greatly increases the osmotic effect, water is withdrawn from the muscle and the weight becomes less. This explanation accounts for the shape of the curve and why it is similar for different salts.

The effect of pH.

To determine the effect of solutions of different pH two series were prepared. One gave pH's from 3 to 8 and was composed of mixtures of sodium hydroxide and phosphoric acid, the proportions necessary to give the desired pH being determined by electrometric titration. The other series gave pH's from 8.3 to 10.5 and was composed of mixtures of sodium carbonate and sodium bicarbonate, the amounts being taken from a titration curve in Folin's Laboratory Manual of Biological Chemistry. The results are given in Table 7.

Table 7.

Percentage weight changes.

pH	Normality				
	0.0	0.13	0.25	0.50	1.00
3	26.7	-1.6	-3 6	-3.0	-16.2
4	28.8	5.1	2.0	-1.5	-10.1
5	28.8	9.5	8.2	4.0	- 6.2
6	28.8	12.5	10.6	26.3	7.9
7	28.8	13.7	34.5	19.6	9.7
8	26.7	30.9	27.8	(saturated)	
8.3	28.6	20.4	20.9	36.4	23.8
9.5	25.4	44.4	52.7	43.9	27.0
10.5	25.4	40.5	36.1	32.5	22.4

These results are shown in Figure 2. It will be seen that at a pH between 5 and 9 the curve is similar to that produced by a salt. At a pH below 5 the muscle acts as if an osmotic effect alone was acting, that is, the solubility of the protein does not seem to affect the weight. Above a pH of 9, the solubility appears to be very marked, a maximum point appears, but there is no minimum. Apparently the solubility of the protein is changed by the pH. J. F. Logan (6) and S. A. Beatty (7) found that fish muscle proteins existed with pH's of 5 and 6 at their isoelectric point. This would account for the sudden change in the shape of the curves at a pH of 5.

The relation between the solubility of the protein and the change in weight.

An experiment was carried out with some fish in which the amount of soluble protein had been determined by Mr. W. W. Johnston at the Experimental Station during the summer 1930. The results are shown in Table 8. As these fish were frozen the values at the maximum point are much greater. Higher values were always found when the fish had been frozen before immersion.

Table 8.
Percentage weight changes

Nitrogen in grams per gram of fish	Normality of solutions			
	0.0	0.15	0.63	1.25
0.0052	18.3	18.5	63.0	48.4
0.0064	19.2	12.6	84.8	62.7
0.0025	23.2	1.7	9.1	11.7
0.0027	11.8	-2.7	2.9	7.0

From these figures it will be seen that the higher values at the maximum point occur with fish which have a greater amount of soluble nitrogen. This would agree with the suggestion that the maximum point is due to the solubility of the protein producing a force great enough to overcome the osmotic force.

The effect of substances which affect the solubility of the protein.

It was decided to try experiments with urea and creatine to see if they had any influence on the solubility of the proteins which might affect the weight change. Merck's white label urea and Eastman's Creatine Hydrate were used. Glycerine and sucrose were also tried. The results are given in Table 9.

Table 9.

Percentage weight changes.

	Percentage concentration of solutions.								
	0.0	0.25	0.5	1.0	2.0	4.0	8.0	16.0	32.0
Urea	22.3	--	--	22.2	22.9	24.8	20.4	38.2	36.8
Creatine	23.2	23.2	24.9	26.2	27.1	--	--	--	--
Glycerine	27.0	--	--	23.9	28.3	25.1	21.0	16.7	6.6
Sucrose	27.0	--	--	30.4	24.3	22.4	24.3	18.5	6.6

It will be seen that with urea there is a slight maximum point at a concentration of 16%; with creatine a slight rise throughout the concentration range. It was impossible to use higher concentrations than 2% on account of the limited solubility. Glycerine and sucrose did not show a behaviour much different from pure water except that they both fell off after a concentration of 8%. No explanation can be offered for these results except the suggestion that the solubility of the protein is affected enough to overcome the osmotic force until very high concentrations, but not enough to cause a rise in weight.

The effect of nitrates and nitrites.

Experiments were carried out with sodium nitrite and potassium nitrate to see if inorganic nitrogen compounds had any effect. The results are given in Table 10.

Table 10.
Percentage weight changes.

	Normality of solutions.					
	0.0	0.125	0.25	0.5	1.0	2.0
Sodium nitrite	25.6	18.5	15.7	24.1	42.7	41.0
Potassium nitrate	25.6	19.0	22.4	35.9	48.3	38.2

From this table it can be seen that inorganic nitrogen compounds do not act differently from other salts, except that the weight change at a normality of 2.0 is greater than that found with the salts previously used. There is a possibility that the inorganic radical containing nitrogen may affect the solubility of the protein, but if this were so, one would expect the maximum point to be higher.

The effect of salts of organic acids.

The following salts of organic acids were used: sodium acetate, benzoate, lithium lactate and lactate; and potassium oxalate and tartrate. The results are shown in Table 11.

Table 11.

Percentage weight changes

Salt	Normality of solutions								
	0.0	0.1	0.12	0.2	0.25	0.4	0.5	0.8	1.0
Sodium acetate	24.6	--	12.7	---	9.8	--	10.6	--	24.8
Sodium benzoate	25.3	--	18.4	---	29.9	--	9.4	--	-5.8
Sodium lactate(a)	26.0	--	15.3	---	11.8	--	7.6	--	2.6
Sodium lactate(b)	29.9	13.7	--	10.7	--	10.1	--	4.7	--
Lithium lactate(a)	24.6	--	-3.1	--	-8.3	--	-9.9	--	-17.0
Lithium lactate(b)	29.9	11.5	--	8.9	--	3.5	--	--	--
Potassium oxalate	25.3	--	15.3	--	14.7	--	29.4	--	33.2
Potassium tartrate	25.3	--	18.5	--	12.0	--	26.5	--	39.8

When the results were first obtained it was seen that the salts which were used all gave results similar to the salts of inorganic acids with the exception of the lactates. The curve given by the lactates showed a steady drop with increasing concentration and showed neither a minimum nor a maximum. As the salts were not of C. P. quality their purity was questioned, and it was found that they both had an acid reaction. The sodium lactate was adjusted by means of sodium hydroxide to a pH of about 6 and the lithium lactate was made more pure by repeated crystallization, until it also had a pH of 6. In the table the original salts are designated by (a) and the purified ones by (b). It can be seen that even when these salts have a pH of 6, the curve is quite different than that given by the other salts investigated. This is apparently due to the lactate ion and no explanation can be offered.

The effect of freezing on the weight changes.

Pieces of fish were frozen in brine and in air and then soaked in salt solutions. Weights were determined after freezing and after periods of one, two and three weeks. Weights of the fresh fish before freezing are also given. The results are given in Table 12.

Table 12.

Percentage weight changes

	Normality of solutions.					
Brine Frozen	0.0	0.15	0.31	0.63	1.25	2.50
Before freezing	36.2	20.5	24.0	35.2	26.8	15.8
After freezing	33.4	19.2	26.6	37.6	32.0	18.6
1 week	30.4	9.5	30.2	58.6	55.6	37.6
2 weeks	24.9	11.5	19.5	46.5	38.2	41.0
3 weeks	17.8	5.1	36.8	70.5	72.6	47.2
Air frozen						
Before freezing	36.2	20.5	24.0	35.2	26.8	15.8
After freezing	44.0	24.8	50.2	63.5	40.0	27.3
1 week	29.8	19.8	33.1	40.0	47.1	22.0
2 weeks	48.0	26.1	58.1	47.8	44.5	23.5
3 weeks	42.0	25.3	54.2	43.5	59.4	29.8
Haddock fillet brine frozen 6 mos. to 1 year	3.3	6.3	10.0	53.3	42.5	25.4

During the periods of storage the fish were kept in a cold storage room at -18°C. The results of this experiment are not consistent and nothing can be deduced from them, except that it might be noticed that when the fish are immersed immediately after freezing, those frozen slowly in air show a greater gain in weight than those frozen in brine. This was noticed in some other experiments. The storage does not seem to have a definite effect on the weight change.

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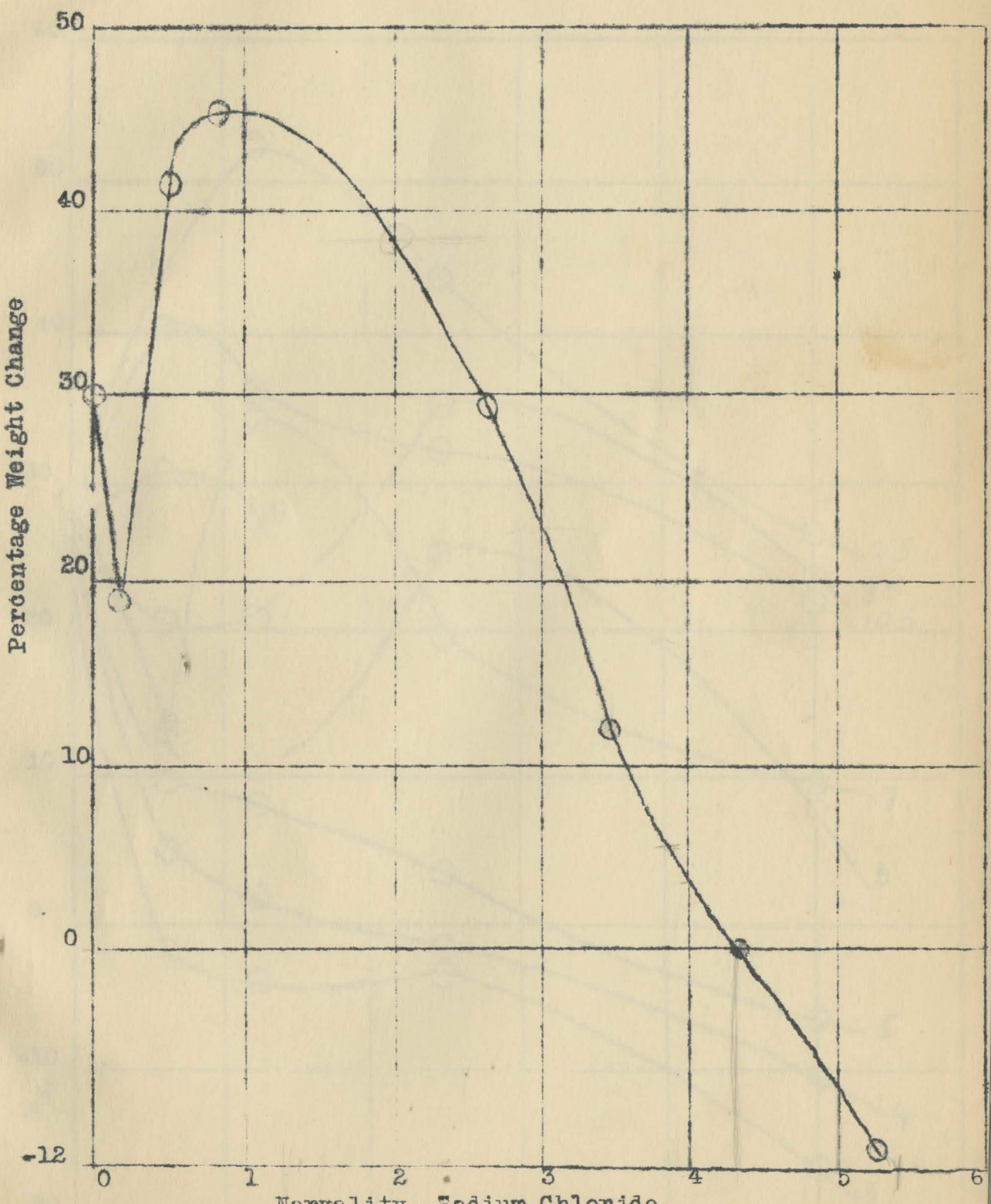
(7) Beatty, S. A. MSS Report to Biological Station 1926.

N. B. The Biological and Experimental Stations referred to in the Bibliography are those operated by the Biological Board of Canada.

Figure I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Percentage Weight Change
0
1
2
3
4
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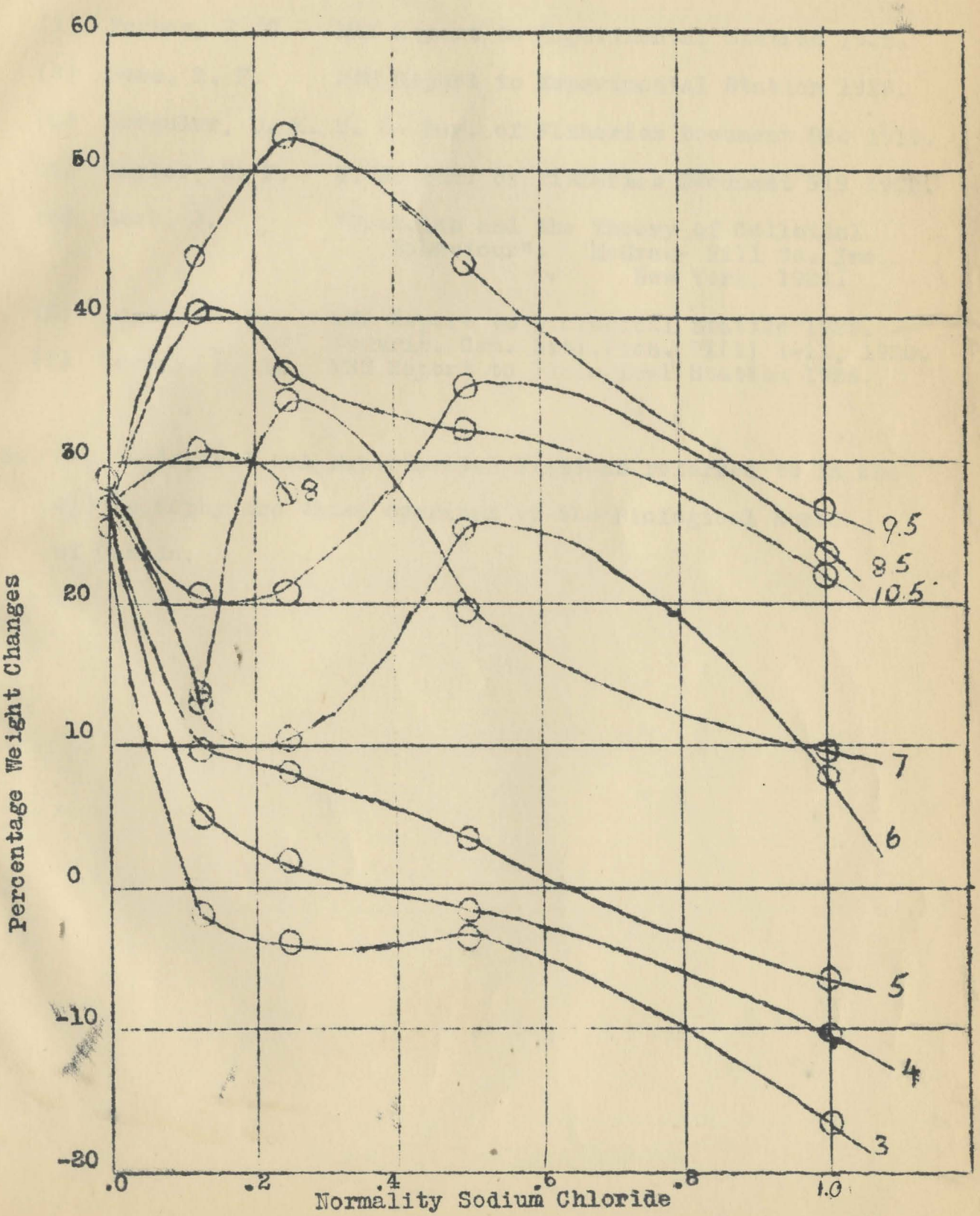
6
Normality Sodium Chloride

Figure II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Percentage Weight Changes
Normality Sodium Chloride
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8.5
10.5
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